Lecture Notes: Biophysics of Macromolecules

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1 Force-extension behavior of linear polymers

In the previous section, we have started from the isotropic elastic rod model and developed several simplified models that could be used to compute the extension of a linear polymer in the absence of external forces. In this section, we will look at the response of linear polymers to an external force F. Starting with the first stretching experiments on DNA by Bustamante and coworkers (1), these models have enabled quantitative comparison between experiments on individual macromolecules and increasingly refined polymer theories of how these molecules respond to external forces. We will start by introducing three models -of increasing sophistication- to describe the stretching response of linear polymers. In all three models, we will initially make several simplifying assumptions:

- We will consider the case that the polymer under study either has no twist rigidity due to the presence of single bonds (as is the case for single-stranded DNA or RNA or many synthetic polymers such as polyethylene glycol) and/or free ends, due to a molecular attachment strategy that allows free rotation at the ends. In this case, the twist and twist-stretch degrees of freedom in the isotropic elastic rod can be neglected, since the twist is zero.

- In addition, we will initially consider a situation where we take the polymer to be inextensible, i.e. it can not be stretched beyond its contour length L_C. This will be true for forces that are small enough that stretching can be neglected, i.e. cases where $F \ll S$, where S is the stretch modulus, in units of force. For double-stranded DNA, $S \approx 1000$ pN (2) and the inextensibility assumption is typically valid for forces ≤ 10 pN.

1.1 Models to describe the stretching response of linear polymers

In the previous sections, we have introduced the freely-jointed chain model, which consists of N segments of length b. The segments are assumed to be perfectly rigid and inextensible. The total length or contour length of the polymer is given by $L_C = N \cdot b$. The segments are further taken to be connected by perfectly flexible hinges or joints. Furthermore, we assume that the segments do not interact and can even pass through each other, i.e. there is no self-avoidance.

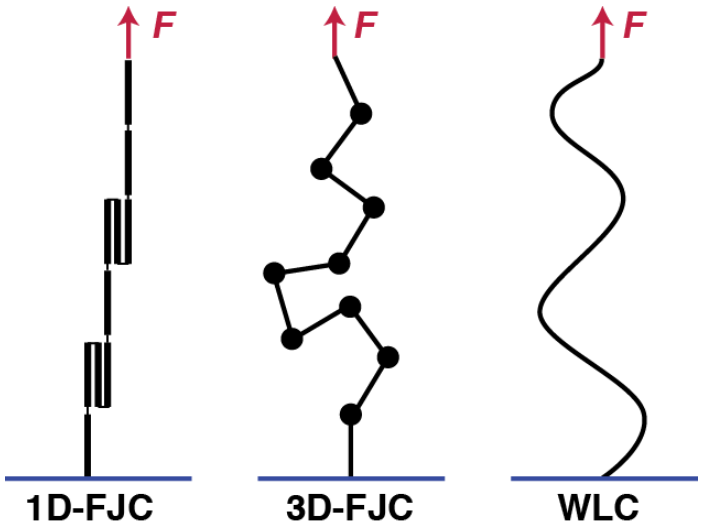

Figure 1: Schematic representation of three models for the force-extension behavior of linear polymers: 1-dimensional freely-jointed chain (1D-FJC), 3-dimensional freely-jointed chain (3D-FJC), and the worm-like chain (WLC). The external applied force is shown as a red arrow.

1.1.1 1-D freely-jointed chain (1D-FJC)

To start, we will take the assumptions of the freely-jointed chain model and further assume that the problem is 1-dimensional, i.e. that the segments are only along one specific directions (Figure 1, left). Let us assume that the segments are all aligned with the z-axis. Each segment can either point "up", along the positive z-direction, or "down", along the negative z-direction. Clearly, this is not necessarily a very realistic model and is only expected to capture the behavior of real polymers in a qualitative fashion. Nonetheless, several key insights emerge from this simple model:

- In the absence of an external force, i.e. for $F = 0$, the polymer just performs a random walk (1D, in this case). We neglect the effect of the presence of the surface and of force transducers such as beads or an AFM cantilever throughout this section. In this case, the mean extension in the absence of force is simply $\langle z \rangle = 0$, as would be expected for a random walk.

- If we apply an external force to stretch the polymer to a length $z > 0$, there is an energy associated with this force given by $-F \cdot z$.

- The polymer resists stretching, i.e. it requires energy to stretch it to an extension z, due to the fact that there are more states or conformations of the polymer for small z than for large z. In particular, if the polymer is stretched to its contour length L_C, there is only one conformation remaining (the one where all segments point along the $+z$ direction). In other words, the polymer resists stretching since It is *entropically* unfavorable to stretch the polymer and this regime is consequently called the *entropic stretching regime*.

The 1D-FJC model is worked out in detail in Phil Nelson's book (3) on page XXX; in addition, there is a nice discussion of it in Philipps, *et al.* (4) on page YYY. The result for the mean extension as a function of the applied force is:

$$\langle z \rangle = N \cdot b \frac{e^{(F \cdot b)/(k_B T)} - e^{(-F \cdot b)/(k_B T)}}{e^{(F \cdot b)/(k_B T)} + e^{(-F \cdot b)/(k_B T)}} = N \cdot b \cdot \tanh(F \cdot b / k_B T) \tag{1}$$

The relationship is graphed in Figure 2.

1.1.2 3-D freely-jointed chain (3D-FJC)

In the previous section, we have assumed that the stiff segments of the FJC model can only point along one direction. However, in a real experimental situation, the polymer can typically move in all three dimensions. So as a next step towards a more realistic model of stretching elasticity, we consider the three-dimensional FJC. There are still N segments (with indices i) of length b that are extensible and connected by perfectly flexible hinges that can now point in any direction. Let us assume that the external forces points into the z-direction and let us introduce a spherical coordinate system where θ denotes the angle with the z-axis. The energy due to the external force is given as a sum over all segments of the projection of the segment length onto the z-axis times the external force:

$$E = \sum_{i=1}^{N} E_i = -F \cdot b \sum_{i=1}^{N} \cos(\theta_i) \tag{2}$$

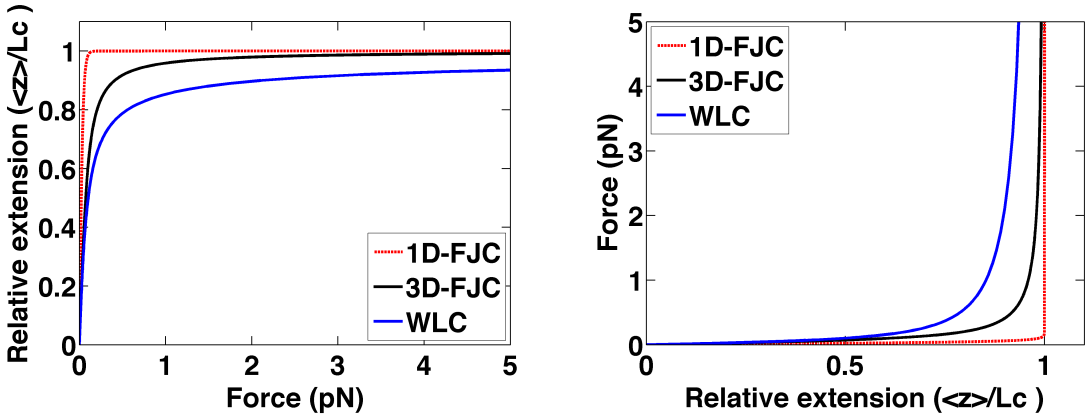

Figure 2: Extension vs. force (left) and force vs. extension (right) predicted by the 1D-FJC, 3D-FJC, and WLC models. The extension has been normalized to the contour length, i.e. we plot $\langle z\rangle/(N\cdot b)$. The segment length was set to $b = 100$ nm for the FJC models and the persistence length was set to $A = 50$ nm for the WLC model.

Since the force is only in the z-direction, we still have a random walk in the x and y-direction and consequently $\langle x\rangle = \langle y\rangle = 0$. We want to compute $\langle z\rangle$ to find the force extension relationship. To this end we will consider the partition function of the 3D-FJC. Recall that the probability of a given configuration of a system is given by its Boltzmann factor, properly normalized:

$$P_j = \frac{\exp(-E_j/k_BT)}{\sum_j \exp(-E_j/k_BT)} = Z^{-1}\exp(-E_j/k_BT) \tag{3}$$

where $\exp(-E_j/k_BT)$ is the Boltzmann factor of state j and $Z = \sum_j \exp(-E_j/k_BT)$ the partition function; the sum runs over all possible states of the system j, in the case of a system that has discrete states (as, for example, the 1D-FJC considered in the previous section). For a system that takes on continuous values, we replace the sum by an integral:

$$Z = \int d\vec{x}\exp(-E(\vec{x})/k_BT) \tag{4}$$

the integration is performed over all relevant variables of the system $\vec{x}$. In our case, each segment can point in any direction on a sphere with radius b. Therefore, the integral takes on the form:

$$Z = \int_0^{2\pi} d\phi_1 \int_0^{\pi} \sin\theta_1 d\theta_1 \ldots \int_0^{2\pi} d\phi_N \int_0^{\pi} \sin\theta_N d\theta_N \exp(-E(\vec{\phi},\vec{\theta})/k_BT) \tag{5}$$

The canonical average of any quantity is computed by averaging the value of the quantity in states j weighted by the probability of the states j. In particular, for the canonical average of z we have

$$\langle z \rangle = \sum_j z_j P_j = Z^{-1} \sum_j z_j \exp(-E_j/k_B T) = Z^{-1} \int d\vec{x} z(\vec{x}) \exp(-E(\vec{x})/k_B T) \tag{6}$$

The extension along z as a function of the variables $\vec{\phi}$ and $\vec{\theta}$ is given by

$$z(\vec{\phi}, \vec{\theta}) = \sum_{i=1}^{N} b \cos(\theta_i) \tag{7}$$

Now, we compute the canonical average for z for the FJC model:

$$\langle z \rangle = Z^{-1} \int_0^{2\pi} d\phi_1 \int_0^{\pi} \sin\theta_1 d\theta_1 \ldots \int_0^{2\pi} d\phi_N \int_0^{\pi} \sin\theta_N d\theta_N \left(\sum_{i=1}^{N} b \cos(\theta_i) \right) \exp(-E(\vec{\phi}, \vec{\theta})/k_B T) \tag{8}$$

In addition, we use the expression for the energy of the system that we found above:

$$\langle z \rangle = Z^{-1} \int_0^{2\pi} d\phi_1 \int_0^{\pi} \sin\theta_1 d\theta_1 \ldots \int_0^{2\pi} d\phi_N \int_0^{\pi} \sin\theta_N d\theta_N \left(\sum_{i=1}^{N} b \cos(\theta_i) \right) \exp\left(\frac{F \cdot b \sum_i \cos(\theta_i)}{k_B T} \right) \tag{9}$$

Now we will use the "stat mech trick" to recognize that the last expression can be written as a derivative of the log of the partition function:

$$\langle z \rangle = k_B T \frac{\partial}{\partial F} \ln(Z) \tag{10}$$

with the partition function

$$Z = \int_0^{2\pi} d\phi_1 \int_0^{\pi} \sin\theta_1 d\theta_1 \ldots \int_0^{2\pi} d\phi_N \int_0^{\pi} \sin\theta_N d\theta_N \exp\left(\frac{F \cdot b \sum_i \cos(\theta_i)}{k_B T} \right) \tag{11}$$

Furthermore, we realize that the partition function is just a product of N identical and independent factors:

$$Z = \left(\int_0^{2\pi} d\phi \int_0^{\pi} \sin\theta d\theta \exp\left(\frac{F \cdot b \cos(\theta)}{k_B T} \right) \right)^N \tag{12}$$

and therefore

$$\langle z \rangle = k_B T \frac{\partial}{\partial F} \ln\left(\left(\int_0^{2\pi} d\phi \int_0^{\pi} \sin\theta d\theta \exp\left(\frac{F \cdot b \cos(\theta)}{k_B T} \right) \right)^N \right) \tag{13}$$

Using basic logarithm rules, we can rewrite as

$$\langle z \rangle = N k_B T \frac{\partial}{\partial F} \ln \left(\int_0^{2\pi} d\phi \int_0^{\pi} \sin\theta d\theta \exp\left(\frac{F \cdot b \cos(\theta)}{k_B T} \right) \right) \tag{14}$$

Next, we do the integral, using the variable substitutions $x = \cos(\theta)$ and $dx = \sin(\theta) d\theta$

$$\langle z \rangle = N k_B T \frac{\partial}{\partial F} \ln \left(\int_0^{2\pi} d\phi \int_{-1}^{1} dx \exp\left(\frac{F \cdot b \cdot x}{k_B T} \right) \right) \tag{15}$$

$$\langle z \rangle = N k_B T \frac{\partial}{\partial F} \ln \left(2\pi \left[\frac{k_B T}{F \cdot b} \exp\left(\frac{F \cdot b \cdot x}{k_B T} \right) \right]_{-1}^{+1} \right) \tag{16}$$

$$\langle z \rangle = N k_B T \frac{\partial}{\partial F} \ln \left(2\pi \frac{k_B T}{F \cdot b} \left(\exp\left(\frac{F \cdot b \cdot x}{k_B T} \right) - \exp\left(-\frac{F \cdot b \cdot x}{k_B T} \right) \right) \right) \tag{17}$$

Next, we do the force-derivative and collect terms:

$$\langle z \rangle = N k_B T F \left(\frac{-1}{F^2} + \frac{b}{F k_B T} \frac{\left(\exp\left(\frac{F \cdot b \cdot x}{k_B T} \right) + \exp\left(-\frac{F \cdot b \cdot x}{k_B T} \right) \right)}{\left(\exp\left(\frac{F \cdot b \cdot x}{k_B T} \right) - \exp\left(-\frac{F \cdot b \cdot x}{k_B T} \right) \right)} \right) \tag{18}$$

Further, using the trigonometric identity

$$\coth(x) = \frac{\exp(x) + \exp(-x)}{\exp(x) - \exp(-x)} \tag{19}$$

we get

$$\langle z \rangle = N k_B T \left(\frac{-1}{F} + \frac{b}{k_B T} \coth\left(\frac{F \cdot b}{k_B T} \right) \right) \tag{20}$$

and finally

$$\langle z \rangle = N \cdot b \left(\coth\left(\frac{F \cdot b}{k_B T} \right) - \frac{k_B T}{F \cdot b} \right) \tag{21}$$

Similar to the 1D-FJC, the 3D-FJC model predicts that initially the polymer can be significantly extended even by relatively small forces; however, as the extension approaches the contour length, higher and higher forces are required to further extend the chain (Figure 2). It is instructive to consider the low and high force limits of the 3D-FJC model.

Low force limit of the 3D-FJC. For low forces, such that $F \cdot b \ll k_B T$, we can expand the coth and use the approximation

$$\coth(\epsilon) \approx \frac{1}{\epsilon} + \frac{\epsilon}{3} + O(\epsilon^3) \tag{22}$$

to obtain

$$\langle z \rangle \approx N \cdot b \cdot \frac{1}{3} \frac{F \cdot b}{k_B T} \tag{23}$$

In other words, solving for F, we find

$$F \approx \frac{3 k_B T}{b^2 N} \langle z \rangle \tag{24}$$

and we can interpret the proportionality constant that connect force and extension as the entropic spring constant: $\frac{3k_BT}{b^2N}$; this is exactly the same result that was previously obtained by considering the fluctuations of a FJC model in the absence of force.

High force limit of the 3D-FJC. For high forces, i.e. in the limit $F \cdot b \gg k_B T$, the coth function approaches 1 and we can write

$$\langle z \rangle \approx N \cdot b \left(1 - \frac{k_B T}{F \cdot b} \right) \tag{25}$$

Again, we can solve the limiting expression for the force and write

$$F \approx \frac{k_B T}{b} \frac{1}{1 - \frac{\langle z \rangle}{N \cdot b}} \tag{26}$$

to see that the force diverges as the extension approaches the contour length $L_C = N \cdot b$. In principle, in this model, it takes an infinite force to extend the polymer to a length that is exactly equal to the contour length. However, in a real experiments, the approximations involved in the model will fail at some point, as will be discussed below.

The 3D-FJC model derived here was used to interpret initial force-extension measurements on double-stranded DNA carried out by Bustamante and coworkers (see Figure 3 and Equation 1 of Ref. (1)). While the DNA stretching data qualitatively behave as predicted by the 3D-FJC model, with a segment length of the order of $b \approx 100$ nm, quantitatively the fit of the model to the data is not great and shows systematic deviations, in particular at intermediate forces. It was found that the WLC model provides a much better fit to the experimental DNA stretching data, as discussed in the next section.

1.1.3 Worm-like chain (WLC)

The worm-like chain model corresponds to the isotropic elastic rod model (REFER TO CORRESPONDING SECTION) in the limit of no twist and no stretch. It models the polymer as an elastic continuum, with a bending stiffness parametrized by the bending persistence length A. The energy of the polymer in this limit is given by

$$E_{bend} = \frac{1}{2} k_B T \int_0^{L_{tot}} (A \beta^2) ds \tag{27}$$

where the integral runs over the contour of the linear polymer. Unfortunately, there is no analytical solution for the extension of the WLC model as a function of force. However, several authors have developed approximation formulae that provide a decent representation of the WLC model and enable a fit of experimental data (5, 6). A commonly used approximation is due to Marko and Siggia (6):

$$\frac{F \cdot A}{k_B T} \approx \frac{1}{4\left(1 - \frac{\langle z \rangle}{L_C}\right)^2} - \frac{1}{4} + \frac{\langle z \rangle}{L_C} \tag{28}$$

An even more precise approximation that employs a seventh-order polynomial is due to Bouchiat *et al.* (7). Again, we can take the low force, low extension limit, for $\langle z \rangle / L \ll 1$ where

$$\frac{1}{\left(1 - \frac{\langle z \rangle}{L_C}\right)^2} \approx 1 + 2\frac{\langle z \rangle}{L_C} \tag{29}$$

and find

$$F \approx \frac{3 k_B T}{2 A L_C} \langle z \rangle \tag{30}$$

Comparing this result to the low force limit of the 3D-FJC (and recalling that $N \cdot b = L_C$), we see again the correspondence $2A \approx b$, i.e. the bending persistence is equal to half the segment length in the corresponding 3D-FJC model. For the experimental stretching data for long double-stranded DNA molecules, it was found that the WLC model describes the data much better and achieves quantitative agreement over the entire force range (8). From the fit, the bending persistence length for double-stranded DNA was determined to be $A \approx 50$ nm.

References

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